

CLAIM AMENDMENTS

1. (Currently amended): An isolated or recombinant nucleic acid comprising (a) a sequence having at least ~~about 70%~~ 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or, (b) a sequence complementary to (a).

2. (Currently amended): An isolated or recombinant nucleic acid of claim 1, comprising a sequence comprising SEQ ID NO:26, ~~and~~ or sequences complementary thereto.

3. (Currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence having at least ~~about 70%~~ 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, ~~and, or~~ (b) sequences complementary to (a), under conditions comprising about 50% formamide at about 37°C to 42°C, 5X SSPE, 0.3% SDS, and 200 n/ml sheared and denatured salmon sperm DNA.

4. (Currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence having at least ~~about 70%~~ 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, ~~and, or~~ (b) sequences complementary to (a), under conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C.

5. (Currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence having at least ~~about 70%~~ 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, ~~and, or~~ (b) sequences complementary to (a), under conditions comprising about 35% formamide at about 35°C to 42°C, 5X SSPE, 0.3% SDS, and 200 n/ml sheared and denatured salmon sperm DNA.

6-15. (Canceled)

16. (Currently amended): An isolated or recombinant nucleic acid comprising (a) at least 30 consecutive bases of a sequence as set forth in SEQ ID NO:26 or, at least 30 consecutive bases of a sequence having at least ~~70%~~ 90% identity to SEQ ID NO:26 and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a).

17. (previously presented): The isolated or recombinant nucleic acid of claim 16, wherein the sequence identity is determined by analysis with a sequence comparison algorithm.

18. (previously presented): The isolated or recombinant nucleic acid of claim 16, wherein the sequence identity is determined by FASTA version 3.0t78 with the default parameters.

19. (Canceled)

20. (Currently amended): The isolated or recombinant nucleic acid of claim ~~19~~ 16, wherein the sequence identity to SEQ ID NO:26 is at least about 95%.

21. (Currently amended): The isolated or recombinant nucleic acid of claim 20, wherein the sequence identity to SEQ ID NO:26 is at least about 97%.

22. (Currently amended): An isolated or recombinant nucleic acid encoding (a) a polypeptide having an esterase activity and having at least ~~70%~~ 90% sequence identity to a sequence as set forth in SEQ ID NO:36, or, (b) enzymatically active fragments of (a).

23. (Currently amended): An isolated or recombinant nucleic acid encoding a polypeptide comprising at least 30 consecutive amino acids of a polypeptide having an esterase activity and having at least ~~70%~~ 90% sequence identity to a sequence as set forth in SEQ ID NO:36.

24-39. (canceled)

40. (previously presented): A method of producing a polypeptide having an esterase activity comprising introducing a nucleic acid as set forth in claim 1 into a host cell under conditions that allow expression of the nucleic acid to produce a polypeptide.

41. (Currently amended): A method of producing a polypeptide having esterase activity comprising at least 30 amino acids of a sequence as set forth in SEQ ED NO:36 or at least 30 amino acids of a sequence encoded by a nucleic acid as set forth in claim 1, comprising introducing a nucleic acid encoding the polypeptide, operably linked to a promoter, into a host cell under conditions that allow expression of the polypeptide, wherein said polypeptide has esterase activity.

42. (previously presented; withdrawn): A method of generating a variant comprising:
obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:26, or a sequence as set forth in claim 1, or, sequences complementary thereto, or fragments comprising at least 30 consecutive nucleotides thereof, or fragments comprising at least 30 consecutive nucleotides of the sequences complementary to SEQ ID NO:26; and
modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

43. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis and any combination thereof.

44. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by error-prone PCR.

45. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by shuffling.

46. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

47. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by assembly PCR.

48. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.

49. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by *in vivo* mutagenesis.

50. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.

51. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.

52. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.

53. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.

54. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by gene reassembly.

55. (original; withdrawn): The method of claim 42, wherein the modifications are introduced by gene site saturated mutagenesis.

56-60. (canceled)

61. (previously presented; withdrawn): A method for comparing a first sequence to a reference sequence wherein said first sequence comprises (a) a nucleic acid sequence as set forth in SEQ ID NO:26 or (b) a sequence having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 or (e) a sequence having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, the method comprising the following steps:

reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

determining differences between the first sequence and the reference sequence with the computer program.

62. (original; withdrawn): The method of claim 61, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

63. (previously presented; withdrawn): A method for identifying a feature in (a) a sequence as set forth in SEQ ID NO:26 or, (b) sequences having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 or (e) having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, the method comprising the following steps:

reading the sequence through the use of a computer program which identifies features in sequences; and

identifying features in the sequences with the computer program.

64. (canceled)

65. (previously presented; withdrawn): A method of catalyzing the hydrolysis of an ester comprising contacting a sample containing an esterase with a polypeptide encoded by a sequence as set forth in claim 1 under conditions which facilitate the hydrolysis of the ester.

66. (canceled)

67. (Currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least ~~about 30, 35, 40, 45, 50, 75, 100, 150 or 200~~ nucleotides in length and having an area of at least 30 contiguous nucleotides of (a) a sequence having at least ~~70%~~ 90% sequence identity to a nucleic acid as set forth in SEQ ID NO:26 or (b) a sequence complementary to (a).

68. (previously presented): The probe of claim 67, wherein the oligonucleotide comprises DNA or RNA.

69-76. (canceled)

77. (Currently amended): The probe of claim ~~[[76 67]]~~, wherein the sequence in (a) has at least 95% sequence identity to the nucleic acid.

78. (previously presented): The probe of claim 77, which is fully complementary to the nucleic acid.

79. (canceled)

80. (original): The probe of claim 67, wherein the probe further comprises a detectable isotopic label.

81. (original): The probe of claim 67, wherein the probe further comprises a detectable non-isotopic label selected from the group consisting of a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.

82. (Currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least about 20 nucleotides in length and having an area of at least 20 contiguous nucleotides of a sequence (a) having at least 90% sequence identity to a nucleic acid as set forth in SEQ ID NO:26, or, (b) its complementary sequence.

83-84. (canceled)

85. (previously presented): A polynucleotide probe for isolation or identification of esterase genes having a sequence which is the same as or fully complementary to at least a portion of SEQ ID NO:26.

86-87. (canceled)

88. (previously presented; withdrawn): A method for modifying small molecules, comprising mixing a polypeptide encoded by a polynucleotide of claim 1 or enzymatically active fragments thereof with a small molecule to produce a modified small molecule.

89. (original; withdrawn): The method of claim 88 wherein a library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity.

90. (original; withdrawn): The method of claim 89 wherein a specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library, and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity.

91. (original; withdrawn): The method of claim 90 wherein the specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated.

92. (original; withdrawn): The method of claim 90 or 91 wherein (a) the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of a small molecule, (b) each biocatalyst is specific for one structural moiety or a group of related structural moieties; and (c) each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

93-96. (Canceled)

97. (Currently amended): An isolated or recombinant nucleic acid comprising at least ~~about 30, 35, 40, 45, 50, 75, 100, 150 or 200~~ consecutive residues of a nucleic acid as set forth in claim 1.

98. (previously presented): A vector comprising a nucleic acid as set forth in claim 1 or claim 23.

99. (previously presented): The vector of claim 98, wherein the vector comprises a viral particle, a baculovirus, a phage, a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

100. (previously presented): A host cell comprising a nucleic acid as set forth in claim 1 or claim 23.

101. (previously presented): The host cell of claim 100 comprising a eukaryotic cell or a prokaryotic cell.

102. (previously presented): The host cell of claim 101 comprising a plant cell, a mammalian cell, a fungal cell, a bacterial cell, a yeast cell or an insect cell.

103-106. (canceled)

107. (Previously presented) The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity comprises catalysis of a transesterification reaction.

108. (Previously presented) The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity comprises catalysis of an acidolysis reaction.

109. (Currently amended): The isolated or recombinant nucleic acid of claim 1, wherein the esterase activity functions at extreme temperatures ~~is thermostable~~.

110. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 35 nucleotides in length.

111. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 40 nucleotides in length.

112. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 45 nucleotides in length.

113. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 50 nucleotides in length.

114. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 75 nucleotides in length.

115. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 100 nucleotides in length.

116. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 150 nucleotides in length.

117. (New): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 200 nucleotides in length.

118. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 35 nucleotides in length.

119. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 40 nucleotides in length.

120. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 45 nucleotides in length.

121. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 50 nucleotides in length.

122. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 75 nucleotides in length.

123. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 100 nucleotides in length.

124. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 150 nucleotides in length.

125. (New): The nucleic acid of claim 97, wherein the oligonucleotide is at least 200 nucleotides in length.